Grant evaluation report

Understanding the ecological niche requirements of rare and endangered orchid species

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Funding requested

The proposed study was focused on understanding the biotic and abiotic niche of a rare orchid *Plantanthera* (Piperia) *cooperi,* native to California Floristic Province. Specifically, $5,050 funding amount was requested from San Diego County Orchid Society. This awarded money helped the project in several ways to accomplish the goals and generate data on the ecology of *P. cooperi*, which can be used in its various conservation programs.

Project Overview

The family Orchidaceae, one of the largest of plant families with an estimated 25,000-30,000 species (Dressler, 1993), is uniquely characterized by an obligatory dependence on Orchid Mycorrhizal Fungi (OMF) for seed germination and development, and partial or complete dependence in later life stages depending on whether the taxa is photosynthetic or non-photosynthetic in adulthood (Girlanda et al., 2011; Mccormick & Jacquemyn, 2014). Orchid distributions are known to be peculiar and generally expected to be a consequence of their extreme preference for unique micro-niches (McCormick, Taylor, Whigham, & Burnett, 2016). Combined together, the trophic dependencies and niche preferences translate into rarity of a majority of orchid taxa if suitable habitat with ambient conditions for orchid seed germination, seedling recruitment and plant growth is not available (Dressler, 1993; Swarts & Dixon, 2009a).

The three prominent fungal families routinely detected in orchids pan-globally include Ceratobasidiaceae, Tulasnellaceae, and Sebacinaceae (Dearnaley, Martos, & Selosse, 2012; Mccormick & Jacquemyn, 2014). Regardless of the host mycobiont interaction, the presence of the appropriate OMF in soil is clearly essential for the sustainability of an orchid population (Swarts & Dixon, 2009b). However, it is less clear that how the abundances of soil OMF that could be utilized by orchid species impact and shape the widespread variation in population size and demography of species within Orchidaceae (Coates,

Lunt, & Tremblay, 2006; Jacquemyn, Brys, & Jongejans, 2010; Rock-Blake, McCormick, Brooks, Jones, & Whigham, 2017; Shefferson, Warren, & Pulliam, 2014).

Regardless of the soil OMF though, root OMF assemblages could be driven by the prerequisite of ambient environment including climatic and soil physicochemical properties of the site (McCormick et al. 2009; Bunch et al. 2013; Mujica et al. 2016). The spatial and temporal variation in abiotic environment imposes selection pressure on orchid host to select or switch to mycobiont, which can perform the mycorrhizal functions. Moreover, the environment directly influences the abundances of OMF in soil, and thus influencing the orchid seed germination and assembly of root OMF (Diez, 2007; McCormick et al., 2012). Altogether, the integration of spatial OMF dynamics and microenvironment surveys in orchid niche might resolve the ambiguities pertaining to orchid distribution and their population dynamics.

In this study, we used a rare orchid *Platanthera* (*Piperia*) *cooperi* (S. Watson) R. M. Bateman (Bateman et al., 2003) native to California Floristic Province as our model species. We specifically selected this Mediterranean taxon because, 1) its populations show contrasting size, and 2) the Mediterranean Domains represent an ideal ecosystem to understand the influence of microhabitat on organisms given their high heterogeneity in physical environment and topographic features. We specifically asked, if *P. cooperi* populations that vary in size have unique identities with respect to root and soil OMF communities, microclimate, and/or soil edaphic characters? We hypothesized that populations of *P. cooperi* with different sizes will host distinct mycorrhizal communities inside roots, and these differences will be tied to the soil mycorrhizal communities and microenvironment of the population.

***Study species*:** *Platanthera cooperi* is a terrestrial, perennial orchid native to California in the United States, and Baja California in Mexico, where its populations occur in scrub, chaparral and woodland ecosystems (Ackerman & Lauri, 2018). In southern California, *P. cooperi* occurs in the San Gabriel Mountain range and peninsular ranges of Los Angeles, San Bernardino, San Diego, Ventura, Orange and Riverside Counties, whereas it is limited to a peninsular range in Baja California (Ackerman & Lauri, 2018). The habitat of *P. cooperi* receives mean annual precipitation of 25cm and has mean minimum and maximum temperatures of 13°C and 21°C, respectively, and its soils vary in texture from sandy clay loam to sandy loam, while the soil pH varies between 5.1 and 8. Plants of *P. cooperi* emerge above ground between December and January with 2-3 basal leaves. Inflorescences can be observed between March and May and bear light green flowers (CNPS, 2018) . Individual flowers are approximately 0.3-0.5cm in diameter (personal observation, Fig. 1). Plant height including the

inflorescence ranges from 14-90cm (CNPS, 2018). The scape bears approximately 100 flowers on the upper 3-56cm of the scape (Ackerman & Lauri, 2018; CNPS, 2018). The flowers of *P. cooperi* have 2.5-9mm long spurs and produce a honey-like fragrance (Ackerman & Lauri, 2018).

Figure 1. Flowering individual of *Plantanthera cooperi*

Study populations: To test our hypotheses, we selected two large and four small populations representing the entire geographic range of *P. cooperi* (Fig. 2). Before finalizing the study locations, we conducted laborious searches at numerous potential *P. cooperi* sites in Orange, Riverside, and San Diego Counties to locate additional populations. These efforts were based on the herbarium records and communication with local and regional orchid experts. While we confirmed one historical location that hosted a few plants, additional large populations were not found. Populations PLF and SCE represented large populations with thousands or hundreds of individuals in a given year, respectively, while the smaller populations (PLE, SCW, CH, and MX) routinely only host between 10 and 30 individuals per populations.

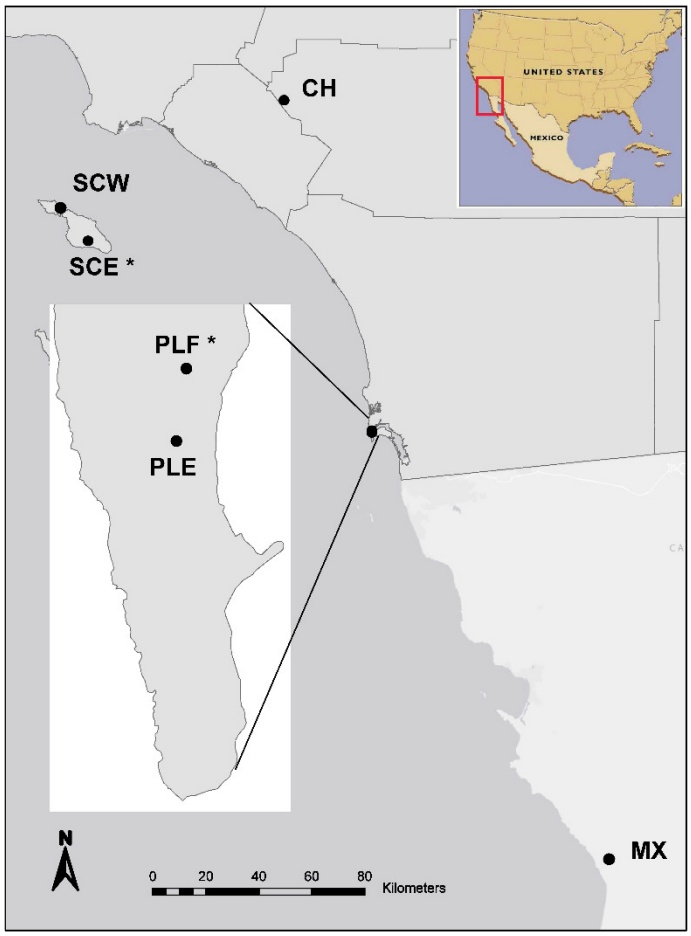


Figure 2. A partial map of United States and Mexico showing the locations of *Platanthera cooperi* study populations. Samples were collected on Point Loma peninsula, San Diego County (PLE, PLF), Santa Catalina island, Los Angeles County (SCE and SCW), Cleveland National Forest, Riverside County (CH), and from one population in Mexico (MX). The * followed by the name of population represent large population size.

Experiment 1: Document the spatial variation in root orchid mycorrhizal fungal communities associated with P. cooperi

To identify the OMF diversity associated with *P. cooperi* roots, we collected roots from seedling, vegetative and reproductive individuals of *P. cooperi* from six study populations across three years. Roots were surface sterilized and DNA was extracted. To assess the fungal diversity inside roots, we amplified and sequenced ITS2 fungal barcoding region of fungal nrDNA.

We observed that Tulasnellaceae and Ceratobasidiaceae OMF families accounted for 95% of the total root fungal communities of *P. cooperi* (Fig. 3). While OMF diversity did not show variation among phenological stages, it showed spatial differences. Two large populations of *P. cooperi*, SCE and PLF, showed higher abundances of Tulasnellaceae in roots when compared to small populations that showed

higher abundances of Ceratobasidiaceae family (Fig. 3). Overall, the OMF communities showed correspondence with population size of *P. cooperi*.

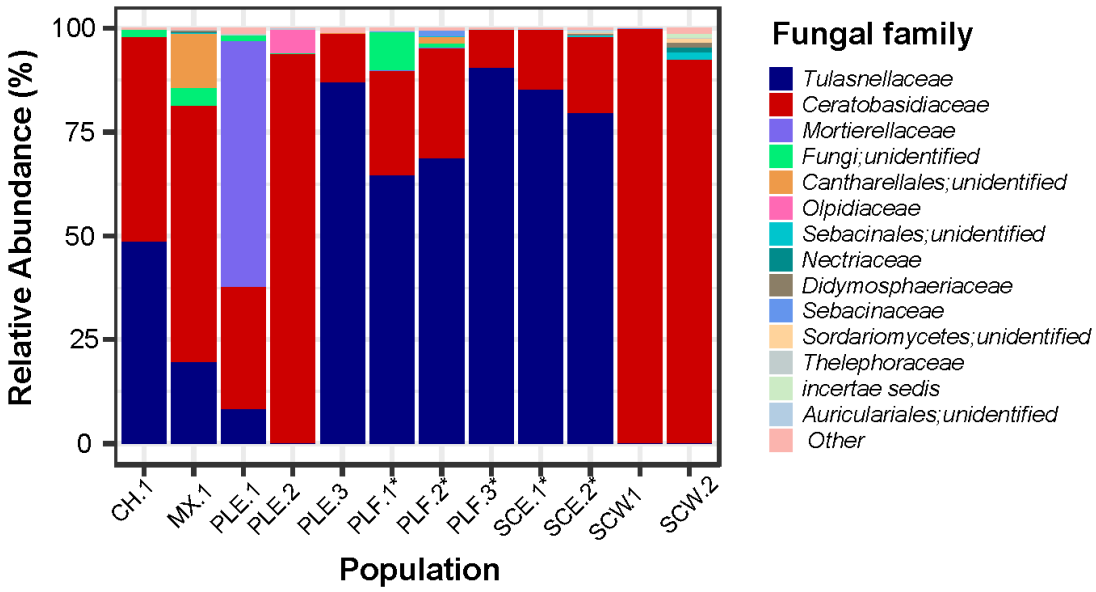
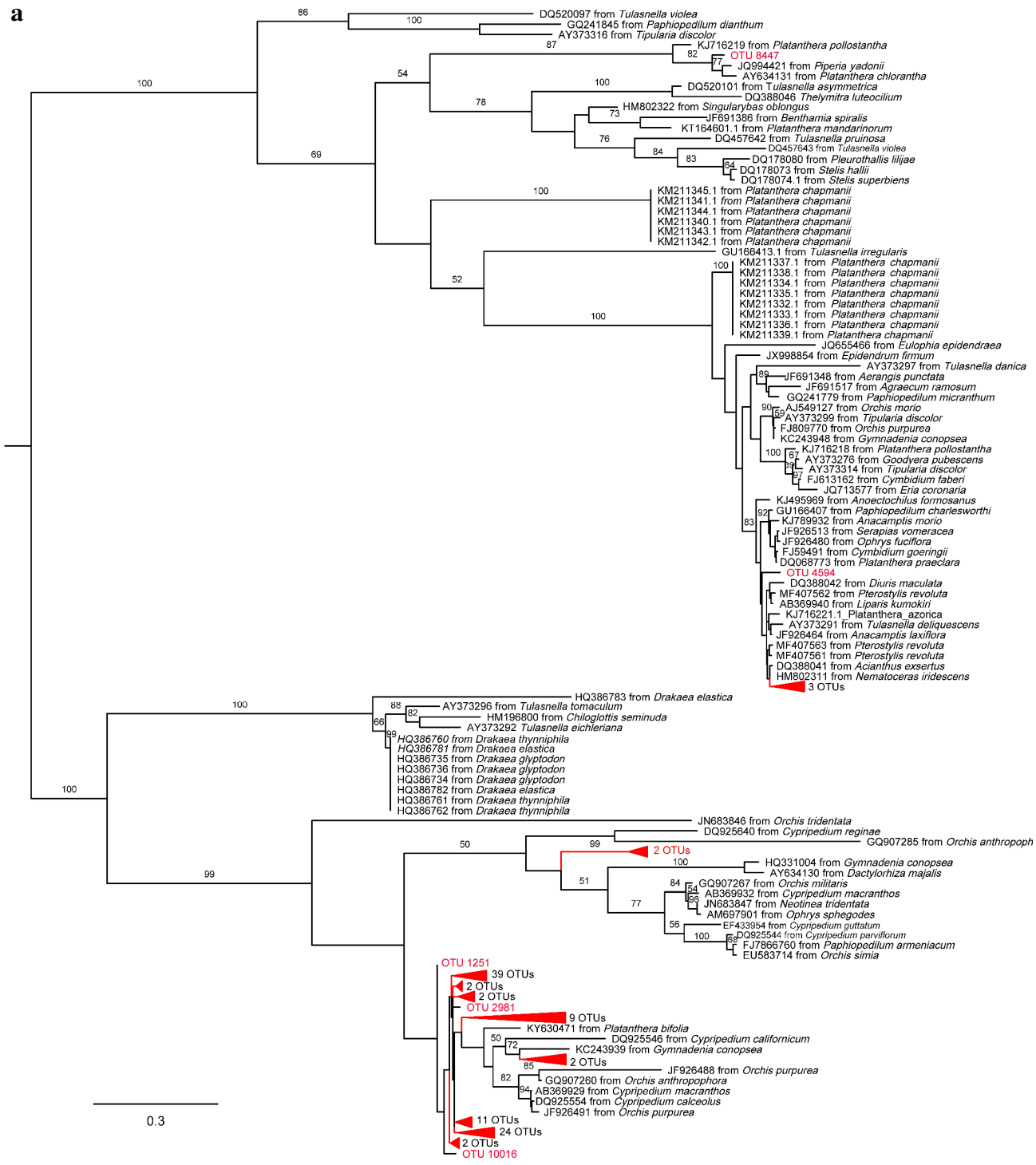


Figure 3. Relative abundances of fungal families identified within roots of *Platanthera cooperi*. The root samples were collected from six populations (PLF, PLE, SCE, SCW, CH and MX), where * symbol followed by the population name represents large population size. The multiple bar for PLF, PLE, SCE and SCW populations represent the fungal communities documented in multiple years.

When we analyzed at the phylogeny of Tulasnellaceae and Ceratobasidiaceae operational taxonomic units (OTUs) identified within roots of *P. cooperi*, we identified that majority of these OTUs made exclusive clades, and did not show close associations with OTUs derived from other orchid species (Fig. 4). In addition, the phylogenetic breadth of Tulasnellaceae OTUs (Fig. 4a) was narrower than Ceratobasidiaceae OTUs (Fig. 4b), suggesting the higher specificity of *P. cooperi* towards Tulasnellaceae.


a
DQ520097 from Tulasnella violea
GQ241845 from Paphiopedilum dianthum
AY373316 from Tipularia discolor
KJ716219 from Platanthera pollostantha
OTU 8447
JQ994421 from Piperia yadonii
AY634131 from Platanthera chlorantha
DQ520101 from Tulasnella asymmetrica
DQ388046 Thelymitra luteocilium
HM802322 from Singularybas oblongus
JF691386 from Benthamia spiralis
KT164601.1 from Platanthera mandarinorum
DQ457642 from Tulasnella pruinosa
DQ457643 from Tulasnella violea
DQ178080 from Pleurothallis lilijae
DQ178073 from Stelis hallii
DQ178074.1 from Stelis superbiens
KM211345.1 from Platanthera chapmanii
KM211341.1 from Platanthera chapmanii
KM211344.1 from Platanthera chapmanii
KM211340.1 from Platanthera chapmanii
KM211343.1 from Platanthera chapmanii
KM211342.1 from Platanthera chapmanii
GU166413.1 from Tulasnella irregularis
KM211337.1 from Platanthera chapmanii
KM211338.1 from Platanthera chapmanii
KM211334.1 from Platanthera chapmanii
KM211335.1 from Platanthera chapmanii
KM211332.1 from Platanthera chapmanii
KM211333.1 from Platanthera chapmanii
KM211336.1 from Platanthera chapmanii
KM211339.1 from Platanthera chapmanii
JQ655466 from Eulophia epidendraea
JX998854 from Epidendrum firmum
AY373297 from Tulasnella danica
JF691348 from Aerangis punctata
JF691517 from Agraecum ramosum
GQ241779 from Paphiopedilum micranthum
AJ549127 from Orchis morio
AY373299 from Tipularia discolor
FJ809770 from Orchis purpurea
KC243948 from Gymnadenia conopsea
KJ716218 from Platanthera pollostantha
AY373276 from Goodyera pubescens
AY373314 from Tipularia discolor
FJ613162 from Cymbidium faberi
JQ713577 from Eria coronaria
KJ495969 from Anoectochilus formosanus
GU166407 from Paphiopedilum charlesworthi
KJ789932 from Anacamptis morio
JF926513 from Serapias vomeracea
JF926480 from Ophrys fuciflora
FJ59491 from Cymbidium goeringii
DQ068773 from Platanthera praeclara
OTU 4594
DQ388042 from Diuris maculata
MF407562 from Pterostylis revoluta
AB369940 from Liparis kumokiri
KJ716221.1 Platanthera azorica
AY373291 from Tulasnella deliquescens
JF926464 from Anacamptis laxiflora
MF407563 from Pterostylis revoluta
MF407561 from Pterostylis revoluta
DQ388041 from Acianthus exsertus
HM802311 from Nematoceras iridescens
3 OTUs
HQ386783 from Drakaea elastica
AY373296 from Tulasnella tomaculum
HM196800 from Chiloglottis seminuda
AY373292 Tulasnella eichleriana
HQ386760 from Drakaea thynniphila
HQ386781 from Drakaea elastica
HQ386735 from Drakaea glyptodon
HQ386736 from Drakaea glyptodon
HQ386734 from Drakaea glyptodon
HQ386782 from Drakaea elastica
HQ386761 from Drakaea thynniphila
HQ386762 from Drakaea thynniphila
JN683846 from Orchis tridentata
DQ925640 from Cypripedium reginae
GQ907285 from Orchis anthropoph
2 OTUs
HQ331004 from Gymnadenia conopsea
AY634130 from Dactylorhiza majalis
GQ907267 from Orchis militaris
AB369932 from Cypripedium macranthos
JN683847 from Neotinea tridentata
AM697901 from Ophrys sphegodes
EF433954 from Cypripedium guttatum
DQ925544 from Cypripedium parviflorum
FJ7866760 from Paphiopedilum armeniacum
EU583714 from Orchis simia
OTU 1251
39 OTUs
2 OTUs
2 OTUs
OTU 2981
9 OTUs
KY630471 from Platanthera bifolia
DQ925546 from Cypripedium californicum
KC243939 from Gymnadenia conopsea
2 OTUs
JF926488 from Orchis purpurea
GQ907260 from Orchis anthropophora
AB369929 from Cypripedium macranthos
DQ925554 from Cypripedium calceolus
JF926491 from Orchis purpurea
11 OTUs
24 OTUs
2 OTUs
OTU 10016
0.3

b

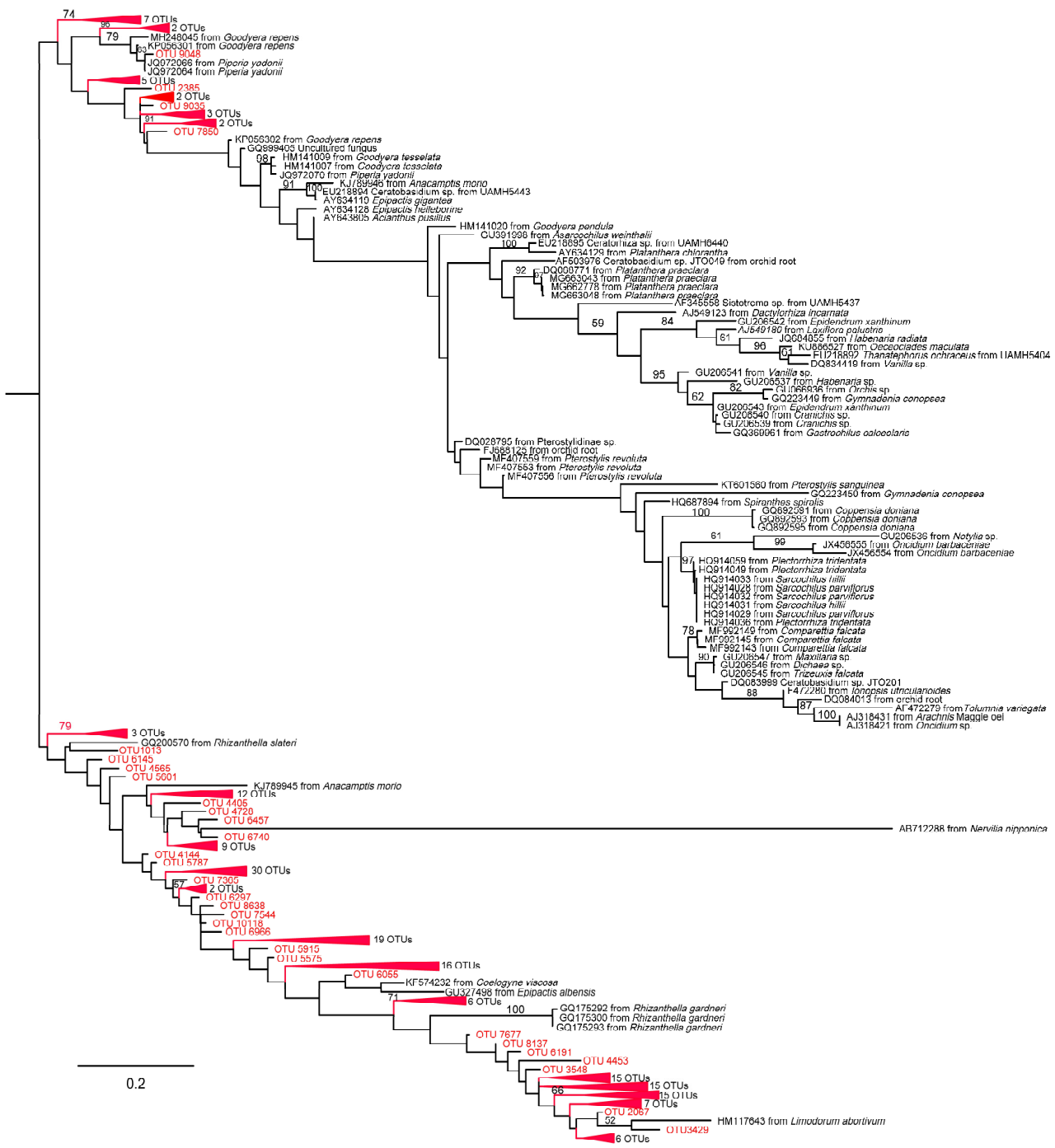


Figure 4. A maximum likelihood tree of, **a)** Tulasnellaceae fungal family, and **b)** Ceartobasdiaceae fungal family generated from internal transcribed spacer 2 (ITS2) locus of nuclear ribosomal DNA. Operational taxonomic units (OTUs) identified within roots of *Platanthera cooperi* and reference sequences from other orchid species or cultures were included. The clades that contained only *P. cooperi* derived OTUs were collapsed, and the number of collapsed OTUs was added in the clade annotation. The tree was mid-point rooted, and only >50 bootstrap values are shown on the tree. The red font or clade indicates the OTUs derived from *P. cooperi.*

Experiment 2: Document the spatial variation in soil orchid mycorrhizal fungal (OMF) communities and their relationship with root OMF communities

We identified the spatial variability in OMF communities associated with roots of *P. cooperi*, thus our next objective was to identify whether OMF communities also show spatial variability in habitat soil of *P. cooperi.* To answer this question, we collected soil cores from six study populations of *P. cooperi* from where roots were collected. To assess OMF diversity in soil, we followed the similar procedures that were used for root OMF communities whereby we amplified and sequenced ITS2 barcoding region of fungal nrDNA.

We observed that Thelephoraceae OMF family showed highest abundance in habitat soil of *P. cooperi* followed by Tulasnellaceae, Agaricaceae, and Ceratobasidiaceae (Fig. 5). In particular, Thelephoraceae abundance was higher at small populations, whereas large populations showed dominance of Tulasnellaceae and Ceratobasidiaceae. Given the dominance of Tulasnellaceae and Ceratobasidiaceae in root fungal communities and their role in explaining the population dynamics of *P. cooperi*, we only selected these two families to compare soil fungal communities.

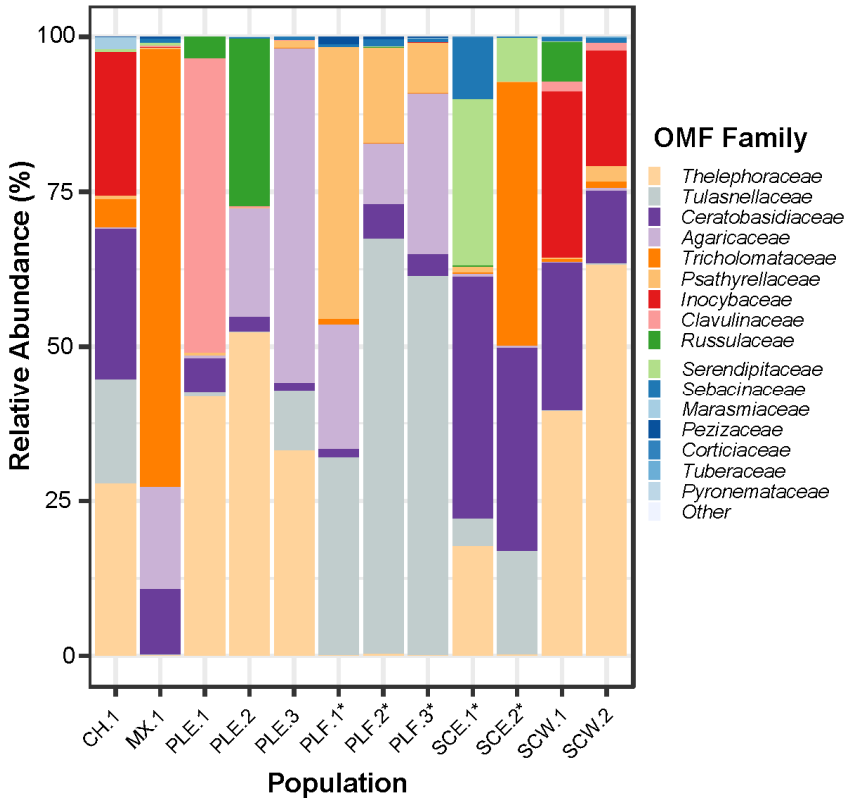


Figure 5. Relative abundances of fungal families identified within habitat soil of *Platanthera cooperi*. The root samples were collected from six populations (PLF, PLE, SCE, SCW, CH and MX), where * symbol followed by the population name represents large population size. The multiple bar for PLF, PLE, SCE and SCW populations represent the fungal communities documented in multiple years.

Similar to root fungal communities, two larger populations, PLF and SCE, grouped close to each other compared to small populations when we examined the Tulasnellaceae and Ceratobasidiaceae OMF families in *P. cooperi* habitat soil. Overall, the relative abundances of soil-associated OTUs showed positive relationship with the same OTU associated with roots. In other words, the OTUs present in higher abundance in roots of large populations were also present in higher abundance in soil of large populations and vice-versa. Overall, a significant overlap between root and soil OMF communities was detected with PCoA and hierarchical clustering that grouped root and soil OMF communities originated from same population together (Fig. 6).

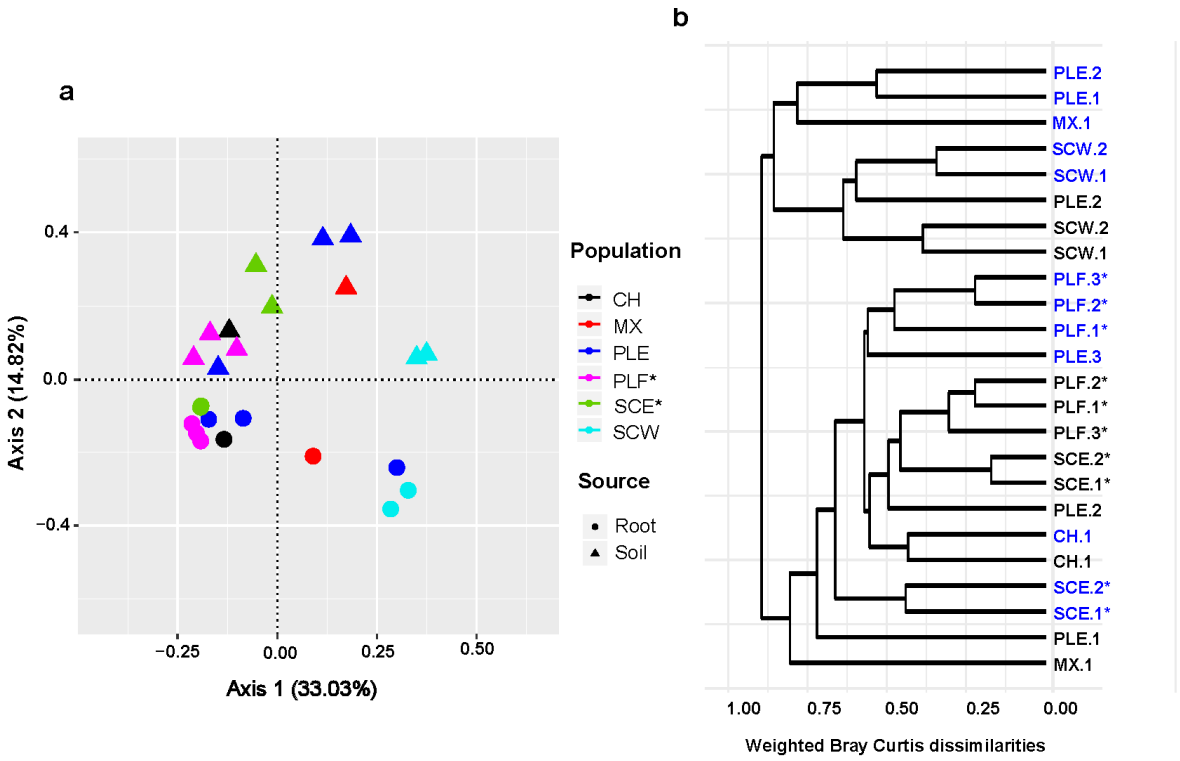


Figure 6. Overlap between root and soil associated Tulasnellaceae and Ceratobasidiaceae fungal communities of *Platanthera cooperi*. The roots and soil samples were collected from six populations (PLF, PLE, SCE, SCW, CH and MX) and the overlap was determined with, **a)** Principle Co-ordinate Analysis, and **b)** hierarchical clustering, where black font represents root samples, whereas blue font represents soil samples. The * followed by the population name represents large populations. The multiple points or nodes for PLF, PLE, SCE and SCW populations represent the data collected across two or three years.

Network analyses with Tulasnellaceae and Ceratobasidiaceae OTUs showed eight components for co-abundance network (SparCC correlation > 0.6 and P < 0.05 for all interactions, Fig. 7a). The first and largest component composed majority of Tulasnellaceae OTUs from soil interacting with Tulasnellaceae OTUs identified inside roots (Fig 7a), while other components consisted of majority of soil Ceratobasidiaceae OTUs interacting with root Ceratobasidiaceae OTUs. Co-exclusion network consisted

of only three components, and in contrast to co-abundance network, it showed the exclusion of Tulasnellaceae OTUs in roots by Ceratobasidiaceae OTUs in soil and vice-versa (SparCC correlation < 0.6 and P < 0.05 for all interactions, Fig. 7b). From 114 Ceratobasidiaceae and Tulasnellaceae OTUs shared between root and soil, the abundances of 73 showed significant positive SparCC correlation between root and soil sources.

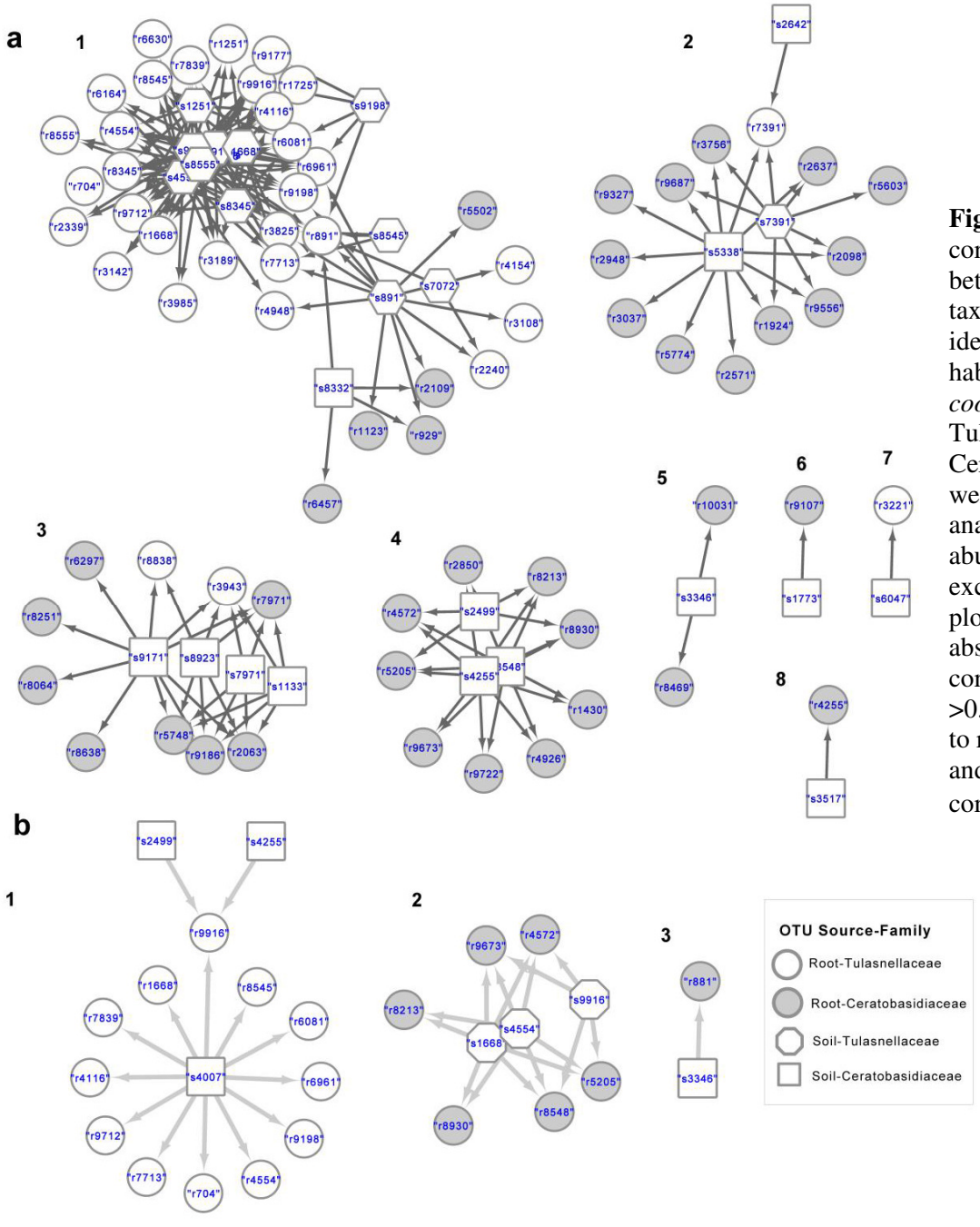


Figure 7. SparCC correlation networks between operational taxonomic units (OTUs) identified from root and habitat soil of *Platanthera cooperi*. Only Tulasnellaceae and Ceratobasidiaceae OTUs were included in the analyses. a) Co-abundance and, b) co-exclusion networks were plotted after filtering for absolute SparCC correlation magnitude of >0.6 and P-value < 0.05 to represent only strong and significant correlations.

Experiment 3: Document the spatial variation in microenvironment

When soil physicochemical profiles were compared across the six study populations, large and small populations showed variation in soil phosphorus (P) concentrations (large = 181 mg/l, small = 32 mg/l, P = 0.01, Table S1).

To explain the variations in OMF communities across the six populations, four variables (Zn, P, OM and silt) identified by forward selection were used in an RDA. The RDA model explained significant variation in OMF communities associated with *P. cooperi* (F = 2.16, P-value = 0.01) roots and soil (Fig. 8). Only RDA1 was identified as the significant axis (F = 4.7, P-value = 0.03) explaining 30% of the variation in root and soil OMF communities. Sites with higher amounts of P and lower amounts of Zn (SCE and PLF) showed higher abundances of Tulasnellaceae family in comparison to Ceratobasidiaceae family.

Of the four measures of microclimate, soil temperature was higher in small populations (18°C) when compared to large population (17°C; P < 0.05, Table S2). The RDA model comprising of air temperature, soil temperature, relative humidity and precipitation, on the other hand, failed to explain the variation in root and soil OMF communities (F = 1.85, P-value = 0.07).

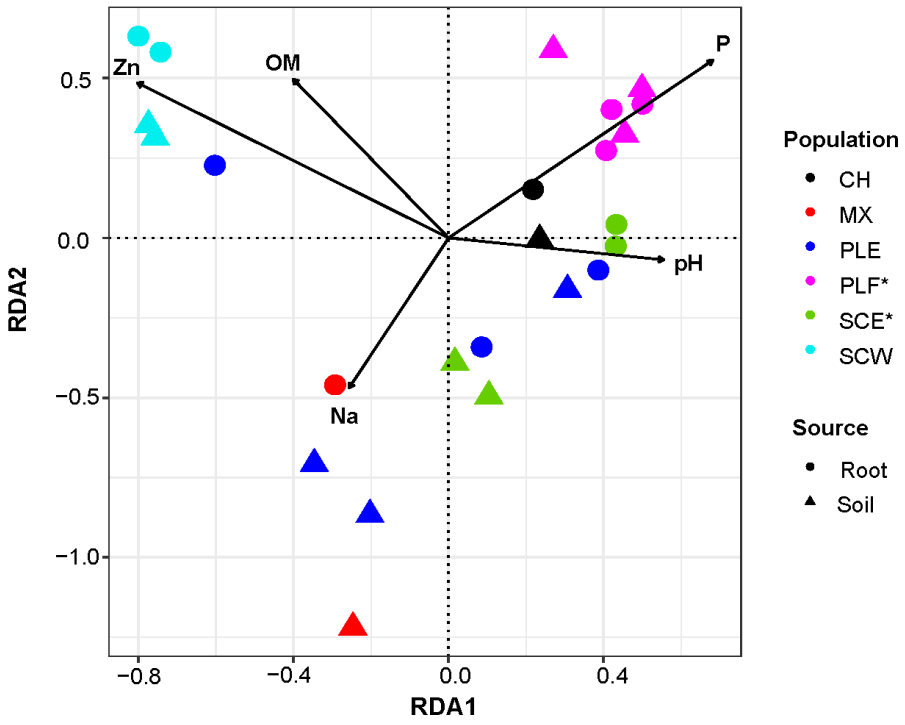


Figure 8. Redundancy analyses (RDA) with Ceratobasidiaceae and Tulasnellaceae fungal communities identified within roots and habitat soil of *Platanthera cooperi* with respect to forwardly selected soil physicochemical variables across six populations (PLE, PLF, SCE, SCW, CH, MX) of *P. cooperi*. The * followed by the population name represents large population size. The multiple points for each population represent the data collected across two or three years.

Table S1. Mean organic matter (OM), cation exchange capacity (CEC), pH, phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), nitrate (NO_3), sulphur (S), zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), boron (B), sand, silt, and clay in soil at study sites. Soil samples were collected from six populations (PLF, PLE, SCE, SCW, CH and MX) in February and April in 2015, 2016 and 2017. Data from replicate samples collected across years were pooled. Kruskal-Wallis non-parametric test was used to compare soil physiochemical profiles of population size groups and study sites.

Population	Population size	n	OM	CEC	pH	P	K	Mg	C	Na	NO_3	S	Zn	Mn	Fe	Cu	B	Sand	Silt	Clay
			%	meq/100g							mg/l								%	
PLF	Large	6	4	18	6.2	295 b	216	331	2452 a	78	4.1	6.4	2 ab	20	37	0.44	0.86	70 ab	13 ab	16
SCE	Large	4	2	12	6.7	50 ab	182	432	1350 ab	58	7.8	4.4	3 ab	10	17	0.65	0.83	48 b	36 b	16
PLE	Small	5	2	7	5.5	27 a	140	335	300 b	94	6.2	6.5	1 a	10	36	0.38	0.53	82 a	6 a	12
SCW	Small	4	6	18	5.5	20 a	239	844	1002 ab	81	8.0	5.8	41 b	36	51	1.33	0.83	57 ab	27 ab	16
MX	Small	3	5	17	6.4	31 ab	432	605	1711 ab	124	4.0	5.9	2 ab	40	39	0.67	1.20	55 ab	27 ab	18
CH	Small	2	7	23	7.0	74 ab	415	415	3538 a	42	12.5	6.5	4 ab	21	18	1.10	1.55	53 ab	25 ab	2
Population P-value			0.17	0.13	0.36	**0.03**	0.54	0.14	**0.04**	0.07	1.00	1.00	**0.03**	0.28	0.26	0.08	1.00	**0.02**	**0.03**	0.58

Table S2. Microclimatic variation in *Platanthera cooperi* habitat. Mean air temperature, relative humidity, soil temperature, and precipitation across four populations (PLF, PLE, SCE and SCW) between December and April across three years.

Population	Population size	Air temperature (°C)	Relative humidity (%)	Soil temperature (°C)	Precipitation (mm)
PLF	Large	17	67	17	0.20
SCE	Large	15	65	16	0.21
PLE	Small	16	65	17	0.19
SCW	Small	16	68	18	0.20
Population P-value		**0.00**	1.00	**0.00**	1.00
Population size P-value		0.06	1.00	**0.00**	1.00

Summary and significance of the findings

We investigated the abiotic and abiotic niche of a rare orchid, *Plantanthera cooperi,* across its natural habitat within Mediterranean climate of California Floristic Province in order to understand the variation in its population size. We identified that Tulasnellaceae and Ceratobasidiaceae OMF families dominated the root fungal communities of *P. cooperi,* where Tulasnellaceae was more abundant in roots and habitat soil of large populations whereas Ceratobasidiaceae was in higher abundance in roots in habitat soil of small populations. The soil physicochemical properties of the large and small populations significantly explained the variation in OMF communities.

The findings from this study could potentially guide the conservation programs focused on *P. cooperi*. The suitable mycobionts can be selected for the introduction programs by taking into consideration the soil physicochemical properties and microclimate of the introduction site. In addition, the observations from this study also helped us to generate new hypotheses on the fundamental ecology of orchids that can be experimentally tested by future research.

References

Ackerman, J. D., & Lauri, R. K. (2018). Piperia cooperi, in Jepson Flora Project (eds.) Jepson eFlora.

Bateman, R. M., Hollingsworth, P. M., Preston, J., Yi-Bo, L., Pridgeon, A. M., & Chase, M. W. (2003). Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). *Botanical Journal of the Linnean Society*. https://doi.org/10.1046/j.1095-8339.2003.00157.x

Bunch, W. D., Cowden, C. C., Wurzburger, N., & Shefferson, R. P. (2013). Geography and soil chemistry drive the distribution of fungal associations in lady's slipper orchid, *Cypripedium acaule*. *Botany*, *91*(12), 850–856. https://doi.org/10.1139/cjb-2013-0079

CNPS. (2018). Inventory of Rare and Endangered Plants of California (online edition, v8-03 0.39). Retrieved September 19, 2018, from http://www.rareplants.cnps.org/detail/2012.html

Coates, F., Lunt, I. D., & Tremblay, R. L. (2006). Effects of disturbance on population dynamics of the threatened orchid Prasophyllum correctum D.L. Jones and implications for grassland management in south-eastern Australia. *Biological Conservation*. https://doi.org/10.1016/j.biocon.2005.06.037

Dearnaley, J. D. W., Martos, F., & Selosse, M. A. (2012). Orchid mycorrhizas: Molecular ecology, physiology, evolution and conservation aspects. In *Fungal Associations, 2nd Edition*. https://doi.org/10.1007/978-3-642-30826-0_12

Diez, J. M. (2007). Hierarchical patterns of symbiotic orchid germination linked to adult proximity and environmental gradients. *Journal of Ecology*, *95*(1), 159–170. https://doi.org/10.1111/j.1365-2745.2006.01194.x

Dressler, R. L. (1993). *Phylogeny and Classification of the Orchid Family*. *Modern methods for business research*. https://doi.org/10.1086/418905

Girlanda, M., Segreto, R., Cafasso, D., Liebel, H. T., Rodda, M., Ercole, E., … Perotto, S. (2011). Photosynthetic Mediterranean meadow orchids feature partial mycoheterotrophy and specific mycorrhizal associations1. *American Journal of Botany*, *98*(7), 1148–1163. https://doi.org/10.3732/ajb.1000486

Jacquemyn, H., Brys, R., & Jongejans, E. (2010). Size-dependent flowering and costs of reproduction affect population dynamics in a tuberous perennial woodland orchid. *Journal of Ecology*, *98*(5), 1204–1215. https://doi.org/10.1111/j.1365-2745.2010.01697.x

Mccormick, M. K., & Jacquemyn, H. (2014). What constrains the distribution of orchid populations? *New Phytologist*, *202*(2), 392–400. https://doi.org/10.1111/nph.12639

McCormick, M. K., Lee Taylor, D., Juhaszova, K., Burnett, R. K., Whigham, D. F., & O'Neill, J. P. (2012). Limitations on orchid recruitment: Not a simple picture. *Molecular Ecology*, *21*(6), 1511–1523. https://doi.org/10.1111/j.1365-294X.2012.05468.x

McCormick, M. K., Taylor, D. L., Whigham, D. F., & Burnett, R. K. (2016). Germination patterns in three terrestrial orchids relate to abundance of mycorrhizal fungi. *Journal of Ecology*, *104*(3), 744–754. https://doi.org/10.1111/1365-2745.12556

Mccormick, M. K., Whigham, D. F., O'Neill, J. P., Becker, J. J., Sarah, W., Rasmussen, H. N., … Taylor, D. L. (2009). Abundance and distribution of Corallorhiza odontorhiza reflect variations in climate and ectomycorrhizae. *Ecological Monographs*. https://doi.org/10.1890/08-0729.1

Mujica, M. I., Saez, N., Cisternas, M., Manzano, M., Armesto, J. J., & Pérez, F. (2016). Relationship between soil nutrients and mycorrhizal associations of two Bipinnula species (Orchidaceae) from central Chile. *Annals of Botany*, *118*(1), 149–158. https://doi.org/10.1093/aob/mcw082

Rock-Blake, R., McCormick, M. K., Brooks, H. E. A., Jones, C. S., & Whigham, D. F. (2017). Symbiont abundance can affect host plant population dynamics. *American Journal of Botany*, *104*(1), 72–82. https://doi.org/10.3732/ajb.1600334

Shefferson, R. P., Warren, R. J., & Pulliam, R. H. (2014). Life-history costs make perfect sprouting maladaptive in two herbaceous perennials. *Journal of Ecology*, *102*(5), 1318–1328. https://doi.org/10.1111/1365-2745.12281

Swarts, N. D., & Dixon, K. W. (2009a). Perspectives on orchid conservation in botanic gardens. *Trends in Plant Science*, *14*(11), 590–598. https://doi.org/10.1016/j.tplants.2009.07.008

Swarts, N. D., & Dixon, K. W. (2009b). Terrestrial orchid conservation in the age of extinction. *Annals of Botany*, *104*(3), 543–556. https://doi.org/10.1093/aob/mcp025